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One new species and new records of three species of the genus *Forcipomyia* Meigen, 1818 (Diptera, Ceratopogonidae) from West Bengal, India

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https://zoobank.org/C2918830-3C6F-498D-A71B-16F68FD5E66F

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Abstract

Forcipomyia (*Forcipomyia*) *hispida*, a new species from the Dooars region of West Bengal, is described and illustrated here based on morphological and molecular data. Among the three newly recorded species, *Forcipomyia* (*Dycea*) *hamoni* de Meillon was known from the Eastern Himalayas while *F.* (*Euprojoannisia*) *calamistrata* Debenham & Wirth and *F.* (*E.*) *fuscimana* (Kieffer) were from the Gangetic plains of West Bengal. The supplementary descriptions and illustrations of the known species are also presented here. DNA barcoding of two newly recorded species of the subgenus *Euprojoannisia* Brèthes is also obtained for the first time.

Key Words

Biting midges, Dooars, *Dycea*, *Euprojoannisia*, Gangetic plains, Eastern Himalayas, new records, new species

Introduction

The biting midges of the genus *Forcipomyia* Meigen, 1818 is the second largest speciose group, comprising 1142 species under 36 subgenera. Among them, 76 species, belonging to the 11 subgenera, are from India (Borkent and Dominiak 2020; Pal and Hazra 2022). The females are adapted to different types of food habits such as, haematophagous, haemolymphoghagous, pollinophagous, and necterophagous (Szadziewski 2016). The subgenus *Dycea* Debenham, 1987, one of the smallest subgenera under the genus *Forcipomyia*, includes only 11 species worldwide; among them, three are from the Oriental realm. In 1914, Brèthes established the subgenus *Euprojoannisia*, which currently includes 105 extant species worldwide (Borkent and Dominiak 2020), including 12 Indian species (Saha et al. 2009). Some adult species of this subgenus are important pollinators of some commercial trees such as cao (*Theobroma cacao*) and rubber (*Hevea brasiliensis*) (Kaufmann 1975; Bystrak and Wirth 1978). The subgenus *Forcipomyia* s. str. is the largest one, which includes 323 extant species worldwide; among them, 35 species are from India (Borkent and Dominak 2020). Immature stages are found in semiaquatic and terrestrial habitats, decaying organic matter, rotten banana stems and logs, bromeliads, wet tree holes, wet moss, algae (Bystrak and Wirth 1978), and also found in the holes made by caterpillar at the base of young leaves of the plant (Chan and Linley 1989).

The objective of this article is to describe and illustrate one new species of the subgenus *Forcipomyia*, and three known species under two different subgenera, *Dycea* (01) and *Euprojoannisia* (02), which are new records from India. Initiative has been taken to obtain DNA barcoding of the mitochondrial cytochrome oxidase subunit I (COI) gene of one species of *Forcipomyia* s. str. and two of *Euprojoannisia*.

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Materials and methods

All the specimens were collected in the eastern part of India. The specimens of one species were collected from the eastern Himalaya (a part of Himalaya Biodiversity hotspot), specimens of another species from the Northern plains of West Bengal characterised by alluvial soil, and specimens of another two species from the Gangetic plains of West Bengal, characterised by alluvial soil. They were caught using open light trap with CFL bulb (27W), black Light (26W) and normal filament bulb (100W) and fabricated UV light trap (8W) (Fig. 1) and preserved in 90% ethanol. A small section of tissue from the ventral part of the thorax and one set of legs from one of the collected specimens of all three species was taken out for DNA extraction, using Qiagen DNeasy Blood and tissue kit. Universal primers [LCO1490 (5′–GGTCAACAAATCATAAAGATATTGG–3′) forward primer and HCO2198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) reverse primer (Folmer et al. 1994)] were used for amplification of the COI region, following the protocol of Harrup, 2018. The amplified products were visualised in 1% agarose gel electrophoresis and outsourced for bidirectional Sanger's sequencing (3730 xl DNA Analyzer, Hitachi). The obtained sequences were uploaded to the NCBI GenBank. Remaining body parts of the voucher specimens left after DNA extraction and the other sorted ones were prepared for glass slide mounts after Wirth and Marston (1968). Accession numbers are provided in the species descriptions.

Adult morphology and terminology follow Alwin and Szadziewski (2013), and terminology of antennal sensilla follows Wirth and Navai (1978). All the measurements were obtained in micrometers (μm), except the width and length of wing in millimeters (mm), with ranges followed by the mean value in parentheses when measures were done from more than two individuals and suffixed

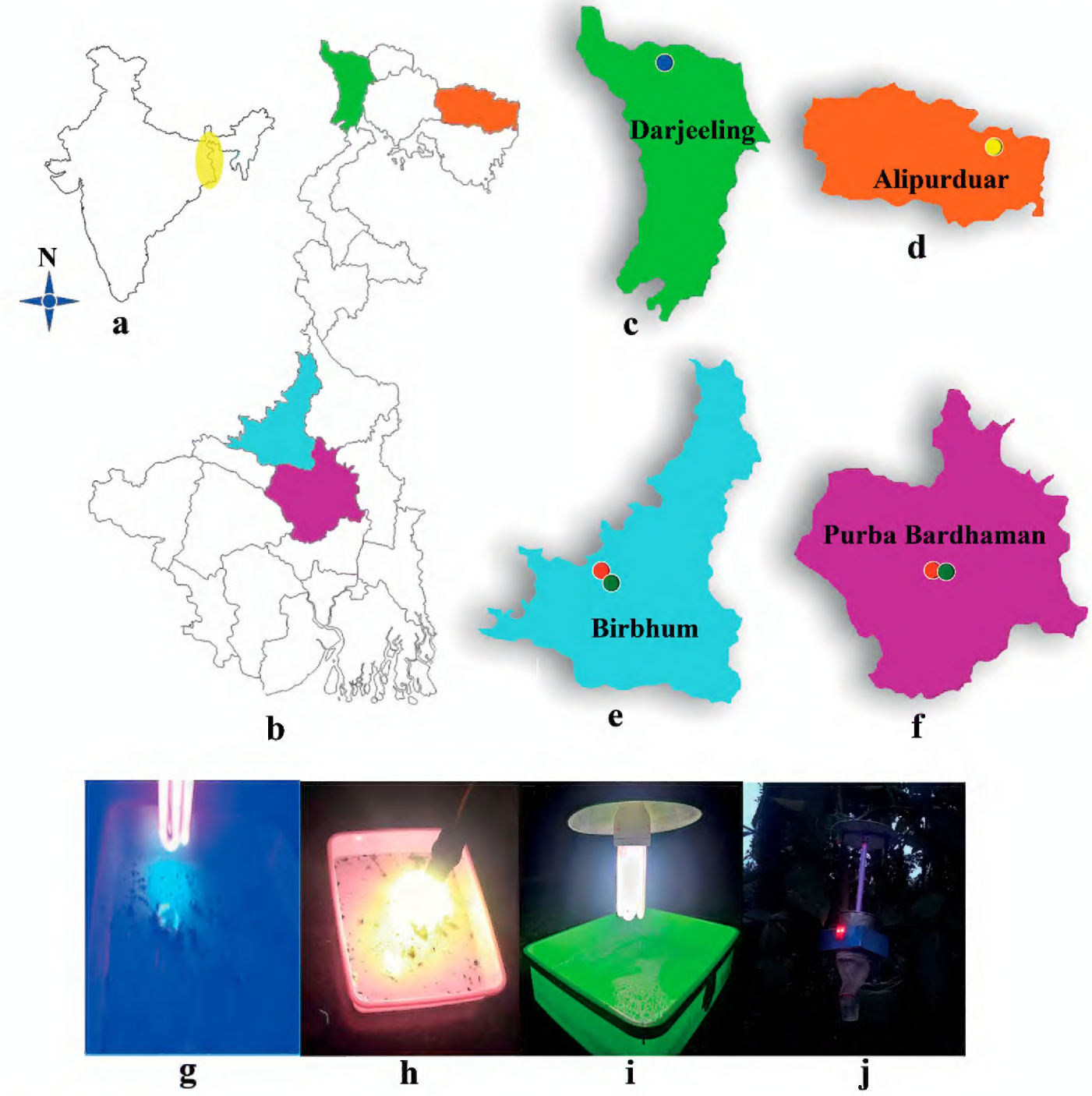

Figure 1. a. Map of India showing the collection state, West Bengal; **b.** Map of West Bengal depicting the collection districts; **c.** Collection site of *Forcipomyia* (*Forcipomyia*) *hispida* sp. nov; **d.** Collection site of *F.* (*Dycea*) *hamoni* de Meillon; **e–f.** Collection sites of *F.* (*Euprojoannisia*) *calamistrata* Debenham and Wirth and *F.* (*Euprojoannisia*) *duscimana* Kieffer; **g.** Open type black light trap; **h.** Open tray halogen bulb light trap; **i.** Open tray CFL bulb light trap; **j.** UV light trap.

by 'n' indicating the number specimens encountered in the study. The proposed new species and newly recorded ones are compared with allied or closely related species following relevant literature (Kieffer 1921; de Meillon 1959; Tokunaga and Murachi 1959; Chan and LeRoux 1971; Debenham and Wirth 1984; Liu and Yu 2001; Saha et al. 2009). The illustrations of specimens were prepared using a compound microscope (Wild Leitz GMBH, Portugal) in combination with an attached Camera Lucida. The photographs were taken under compound microscopes (Leica DM 1000, Germany, and Wild Leitz GMBH, Portugal), using an attached digital camera.

Types and other materials are presently kept at the entomological collection of the Department of Zoology, The University of Burdwan (India), and will be deposited at the National Zoological Collections (**NZCI**) in due course.

Abbreviations with their full forms. **AR** – Antennal ratio, **PR** – Palpal ratio, **TR** – Tarsal ratio, **WL** – Wing length, **WW** – Wing width, **CR** – Costal ratio, **HTC** – Hind tibial comb.

Results

Taxonomy

Subgenus *Forcipomyia* s. str.

Forcipomyia (*Forcipomyia*) *hispida* sp. nov.

https://zoobank.org/9FEBDE1C-113E-45E1-96D0-C41A4E949F74

Fig. 2

GenBank accession number. OP730326.

Type material. ***Holotype*** ♂, India, West Bengal, Alipurduar, Jayanti (26°41'58.56"N, 89°36'49.68"E), Light trap, 12.XI.2021, Col. G.S. Pal. ***Paratypes*** 3♂ and 1♀, same data as holotype.

Diagnosis. The only species of the subgenus *Forcipomyia* may be diagnosed by the following combination of characters: **Adult male.** Gonocoxite long, narrow and curved at middle, apical part of it bearing tuft of setae; gonostylus inflated at middle and curved apically; base of parameres separated, sub–median process of parameres inwardly curved apically. **Adult female.** Distal half of third palpal segment swollen with large sub–apical sensory pit and subequal spermathecae.

Etymology. The specific name "*hispida*" is derived from the tuft of setae at the distal end of the gonocoxite.

Description. Male (n = 4). ***Head.*** Head dark brown, eye contiguous and bare. First and last five flagellomeres longest and terminal flagellomere with terminal papilla (Fig. 2a). Length ratio of flagellomeres (I–XIII): 24–28 (25.5): 13–15 (14): 14–17 (15.7): 14–17 (16.2): 14–16 (15): 16–17 (16.7): 16–18 (17): 16–18 (17.5): 16–18 (17): 65–67 (65.7): 35–40 (38.2): 24–28 (26): 35–38 (36.7); AR 1.38–1.42 (1.40). Third maxillary palp (Fig. 2b) longest, round, shallow sensory pit at sub–apical end. Length ratio of palpal segments (I–V): 12–13 (12.7): 15–17 (16): 35–37 (36.5): 13–14 (13.7): 11–12 (11.5). PR 4.11–5.28 (4.76).

Thorax. Dark brown. Scutellum with nine setae. Wing (Fig. 2c). Covered with numerous microtrichia and sparsely distributed macrotrichia. WL 1.10–1.16 (1.13), WW 0.37–0.39 (0.38), CR 0.45–0.47 (0.46). Legs. Pale brown in colour without any banding pattern. HTC (Fig. 2d) with 8 spines and spur. TR_I 1.30–1.36 (1.32), TR_{II} 1.12–1.16 (1.13), TR_{III} 1.05–1.11 (1.08).

Abdomen. Brown without any scale.

Genitalia (Fig. 2e–i). Sternite IX 110–120 (116) long and 205–212 (208.75) wide, 1.8 times wider than length. Tergite IX 180–186 (182.25) long, 209–215 (212.25) wide and posterior margin rounded not extending beyond half of gonocoxite with long apicolateral process ending with 5–6 setae. Gonocoxite dark brown, slightly arch, middle portion narrower than base and apex, 186–202 (197.5) long, almost 3× its greatest breadth, 62–70 (66.75) with stout setae on surface and tuft of setae at its distal end; gonostylus pale brown, 107–112 (109.75) long and 20–23 (21.5) wide, usually half as long as gonocoxite; swollen medially, distal part slightly curved. Parameres (Fig. 2e) separated; lateral process of parameres well developed extending to base of gonocoxite; sub–median process shorter and apical part little curved inwardly. Aedeagus with (Fig. 2f) little curved basal arms with highly sclerotised, well developed basal arch, with a rod like ridge at middle and triangular apex.

Female (n = 1). Similar to male with usual sexual differences.

Head. All flagellomeres of antenna (Fig. 2j) bearing sensilla chaetica, flagellomeres IX–XIII bearing sensilla trichodea, VI–XII with few sensilla basiconica. Length ratio of flagellomeres (I–XII): 18: 17: 21: 20: 19: 19: 19: 17: 21: 20: 20: 19: 32; AR 0.74. Length ratio of palpal segments (Fig. 2k) (I–V): 13: 20: 41: 09: 11; PR 3.41.

Thorax. Wing (Fig. 2l) large and wide, r_1 cell obsolete and r_2 cell open, WL 1.19, WW 0.47 and CR 0.46. HTC (Fig. 2m) with 9 spines. Tarsomeres of fore legs missing, TR_I not measurable, TR_{II} 1.20 and TR_{III} 1.06.

Abdomen. Dark brown. Spermathecae (Fig. 2n) two, nearly subequal, highly sclerotised measuring about 50× 45 and 65× 58.

Larva and Pupa. Unknown.

Discussion. The new species is more or less similar to the Indian species, *Forcipomyia* (*Forcipomyia*) *imparitheca* Saha, Das Gupta, Gangopadhyay & Mukherjee, 2009 in the shape of aedeagus and maxillary palp but differs significantly in the structure of gonocoxite with tuft of setae, and shorter parameres having shorter submedian process than the known species. The male of the new species is also similar to the Chinese species *F. dividus* Liu & Yu, 2001 in the aedeagal structure, but the shape of the parameres is completely different from each other. The parameres are fused in *F. dividus* while they are separate in the new species; submedian process is much longer, and TR value is much higher in *F. dividus* than the new species.

The females of *F. imparitheca* show resemblances to the new species in AR, PR and CR values. New species possesses subequal spremathecae without neck while in *F. imparitheca*, the spermathecae are unequal, with short neck. The female of the new species is also similar

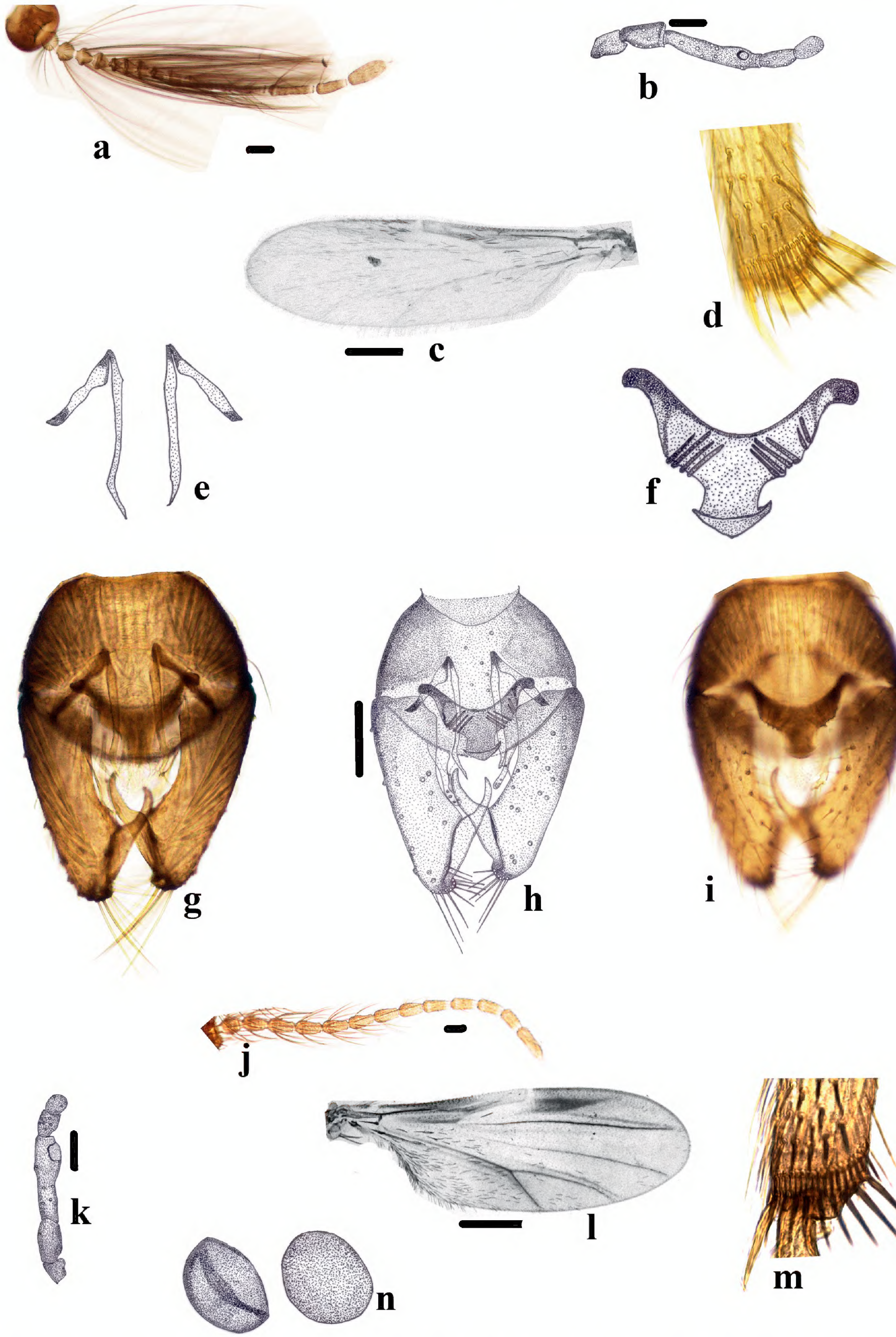

Figure 2. *Forcipomyia* (*Forcipomyia*) *hispida* sp. nov. **a–h** male (♂); **a.** antenna; **b.** maxillary palp; **c.** wing; **d.** hind tibial comb; **e.** parameres; **f.** aedeagus; **g.** photograph of male genitalia (dorsal view); **h.** illustration of male genitalia; **i.** photograph of male genitalia (ventral view); **j–n** female (♀); **j.** Antenna; **k.** maxillary palp; **l.** wing; **m.** hind tibial comb; **n.** spermathecae. Scale bar: 0.05 mm.

to another Indian species, *Forcipomyia fulvipes* Saha, Das Gupta, Gangopadhyay & Mukherjee, 2009 in the shape of spermathecae (subequal), AR and CR values, much lower PR values. However, PR value and shape of the third maxillary palp segment differ from each other (PR value is much lower in *F. fulvipes* than the new species; the third maxillary palp segment in *F. fulvipes* is swollen in the middle but swollen at the distal end in new species).

Distribution. India (West Bengal).

Subgenus *Dycea* Debenham, 1987

Forcipomyia hamoni de Meillon, 1959

Fig. 3

Forcipomyia hamoni de Meillon, 1959: 329; Liu and Yu 2001: 486.

Material examined. 2♂♂, India, West Bengal, Darjeeling, (27°2'51"N, 88°16'6.96"E), Light trap, 09.XI.2021, Col. G. S. Pal.

Diagnosis. Male. Wing densely covered with microtrichia. Aedeagus shield shaped and tip of the aedeagus with small pointed projection. Parameres fused basally and strongly constricted, lateral process well developed and elongated.

Male (n = 2).

Supplementary description. Head dark brown. Eyes contiguous. Length ratio of flagellomeres (I–XIII): 36–38: 22–23: 22: 22: 21: 20–21: 21: 21: 21–22: 68–71: 45–47: 35–36: 47–50; AR 1.18. Length of palpal segments: 10–11: 18–20: 33–36: 21–22: 15–16; PR 3.27–3.30. Wing large with dense microtrichia, WL 1.77–1.81, WW 0.50–0.52, CR 0.44–0.45. TR_I 0.83–0.86, TR_{II} 0.57–0.59 and TR_{III} 0.66–0.68. Gonocoxite almost 2.5 times longer than its greatest width (142.5× 57.5); gonostylus 0.7 times shorter than gonocoxite. Aedeagus (Fig. 3) triangular, shield shaped with pointed projection at tip.

Discussion. Wing length of both African and Chinese specimens is much greater than the Indian counterpart, but the wing width is almost the same, and value of AR and TR_{III} is greater than those of Indian species.

Female, Pupa and Larva. Unknown.

Distribution. Burkina Faso, South Africa, China and India (new record).

Subgenus *Euprojoannisia* Brèthes, 1914

Forcipomyia calamistrata Debenham & Wirth, 1984

Fig. 4

Forcipomyia calamistrata Debenham & Wirth, 1984: 862; Lie and Yu 2001: 495.

GenBank accession number. MZ769938

Material examined. 3♂♂, India, West Bengal, Purba Bardhman, Burdwan Town (23°13'33"N, 87°51'30"E), Light trap, 18.IV.2021, Col. N. Hazra. 2♂♂, West Bengal, Birbhum, Suri, Tilpara (23°56'39.48"N, 87°31'31"E), light trap, 13.XI.2020, Col. G.S. Pal.

Diagnosis. Male. Aedeagus with tooth like serrations at apex and subapex. **Female.** Mandible with 30 teeth; spermathecae two, unequal, large and oval.

Male (n = 5).

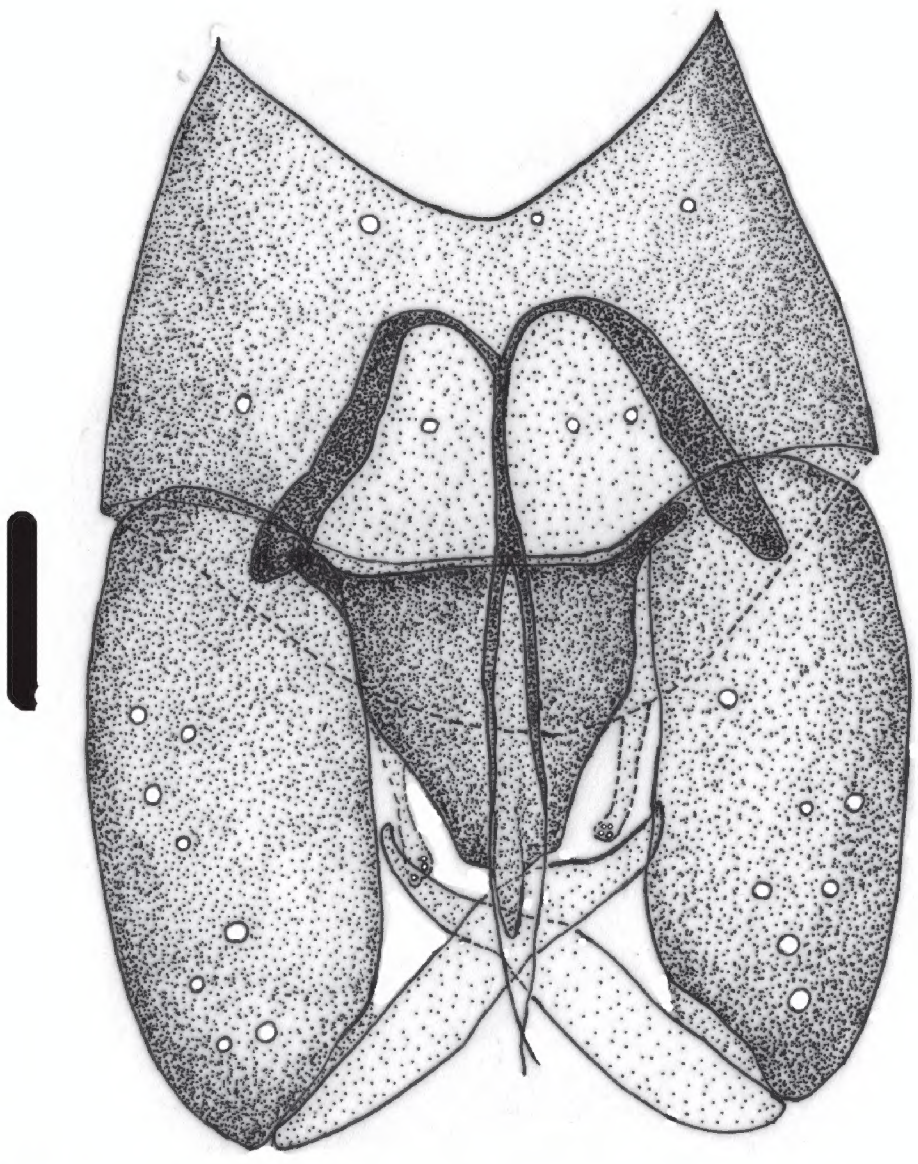

Figure 3. *Forcipomyia* (*Dycea*) *hamoni* de Meillon, 1959. Adult male genitalia. Scale bar: 0.05 mm.

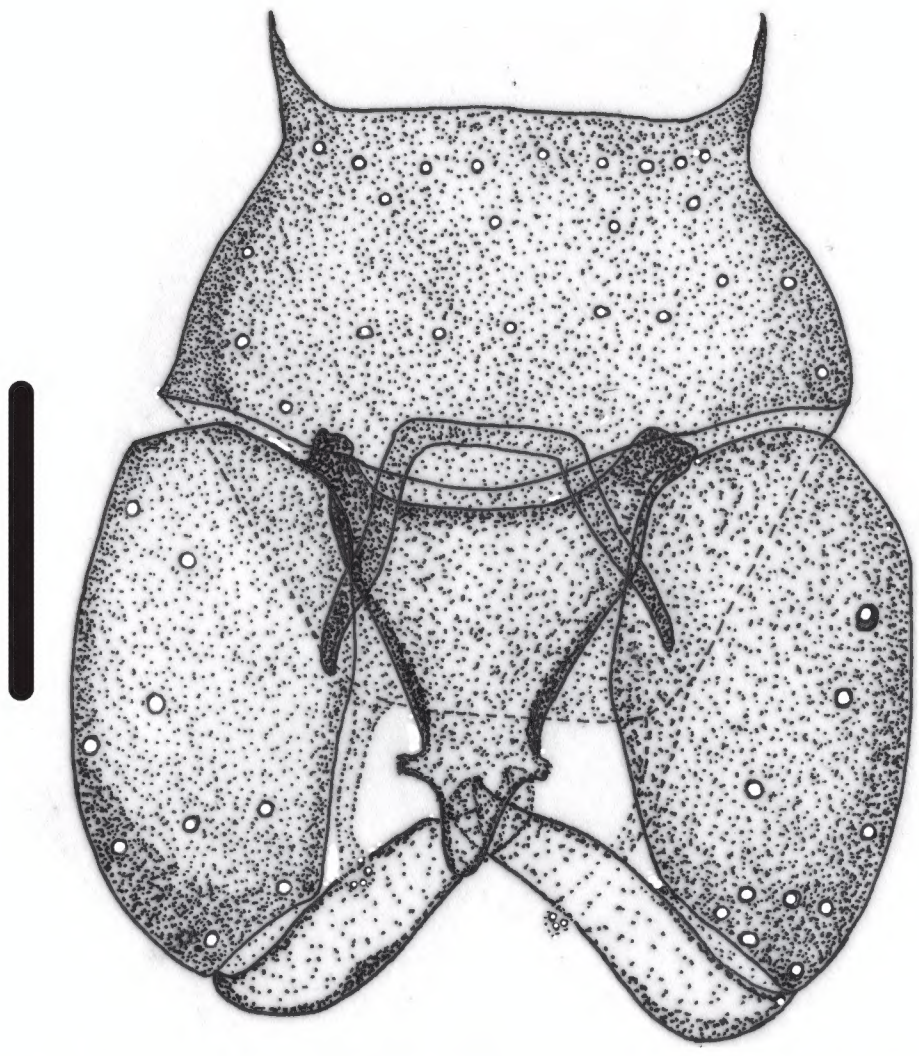

Figure 4. *Forcipomyia* (*Euprojoannisia*) *calamistrata* Debenham & Wirth, 1984. Adult male genitalia. Scale bar: 0.05 mm.

Supplementary description. Head dark brown. Antenna with dark brown second segment and others pale brown; length ratio of flagellomeres (I–XIII): 17–19 (17.6): 12–14 (12.8): 12–14 (13): 12–14 (13): 11–13 (12.2): 11–13 (12): 11–13 (12.4): 13–16 (13.8): 12–14 (13.4): 37–41 (38): 24–27 (25.6): 17–19 (18): 27–30 (29); AR 1.13–1.16 (1.14). Length ratio of palpal segments (I–V): 10–12 (10.6): 12–14 (13.2): 24–29 (26.6): 14–16 (15.4): 8–10 (8.8); PR 3.57–3.85 (3.72). WL 0.85–0.88 (0.86), WW 0.30–0.33 (0.32); CR 0.39–0.43 (0.4). TR_I 0.81–0.87 (0.84), TR_{II} 0.85–0.92 (0.87), TR_{III} 1.35–1.41 (1.38). Gonocoxite almost twice as long as wide bearing numerous setae, broad in middle and constricted at apex with well developed apicolateral process. Gonostylus little shorter than gonocoxite. Aedeagus almost triangular, basal arch shallow with apical and subapical serrations (Fig. 4).

Discussion. Both palpal and hind tarsal ratios are lower than those of Chinese ones.

Distribution. China, Taiwan, Australia and India (new record).

Forcipomyia fuscimana (Kieffer, 1921)

Fig. 5

Forcipomyia fuscimana (Kieffer, 1921): 559; Tokunaga and Murachi 1959: 216.

Forcipomyia pennielongata Chan & LeRoux, 1971: 733; Liu and Yu 2001: 511.

GenBank accession number. MZ539943

Material examined. 2♂♂, India, West Bengal, Purba Bardhman, Burdwan Town (23°13'33"N, 87°51'30"E), Light trap, 09.I.2021, Col. N. Hazra. 2♂♂, West Bengal, Birbhum, Suri, Maliha (23°51'11.76"N, 87°32'58"E), Light trap, 16.XI.2020, Col. G. S. Pal.

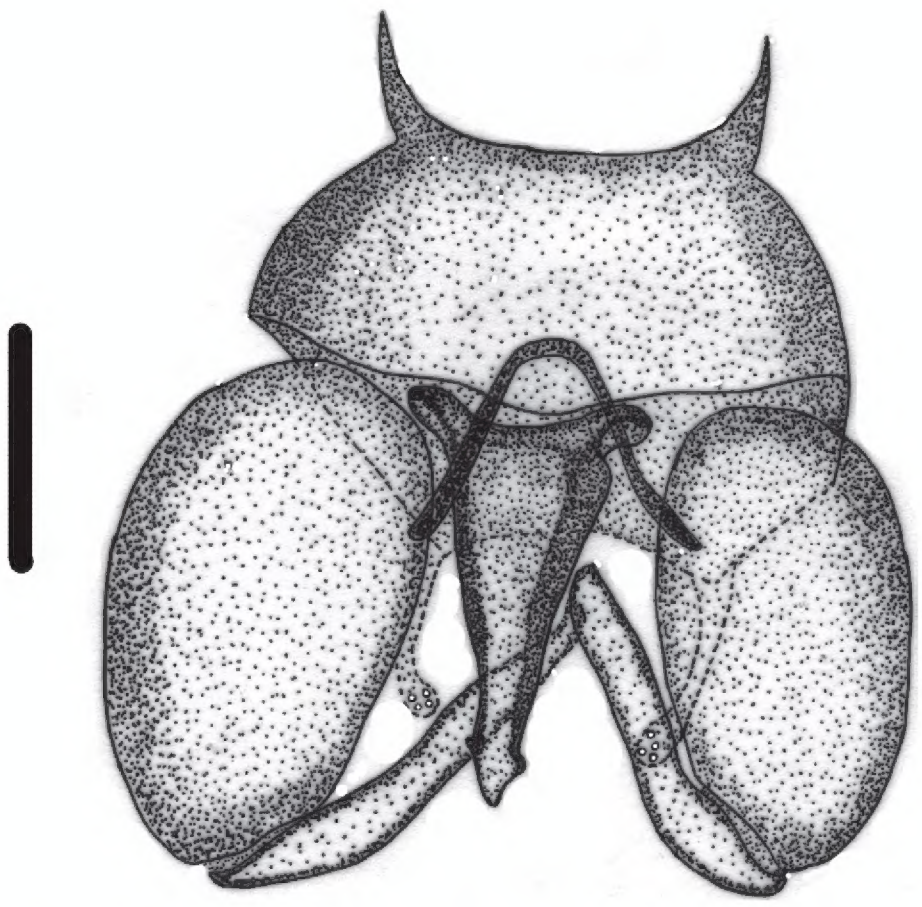

Figure 5. *Forcipomyia* (*Euprojoannisia*) *fuscimana* (Kieffer, 1921). Adult male genitalia. Scale bar: 0.05 mm.

Diagnosis. Male. Aedeagus narrowly 'V' shaped, basal arch much broader than apical part. **Female.** Third maxillary palp segment with irregular sensory pits bearing capitate sensilla. Spermathecae two, subequal and oval.

Male (n = 4).

Supplementary description. Length ratio of flagellomeres (I–XIII): 30–32 (30.7): 17–18 (17.5): 18–20 (19.5): 20–21 (20.7): 16–17 (16.5): 16–18 (16.7): 18–19 (18.2): 19–22 (20.7): 21–25 (23.5): 59–63 (61): 42–46 (42.5): 24–27 (25.2): 30–32 (31); AR 1.12–1.15 (1.13). Length ratio of palpal segments (I–V): 12–13 (12.2): 13–15 (14): 36–41 (38.2): 21–24 (22.7): 10–12 (11); PR: 4.50–5.12 (4.78). WL 1.15–1.20 (1.17), WW 0.40–0.43 (0.42); CR 0.44–0.47 (0.45). Tarsal ratio of fore, mid and hind legs 0.93–0.98 (0.96), 0.57–0.61 (0.59), 0.65–0.71 (0.68) respectively. Gonocoxite 1.75 times longer than width, gonostylus about 0.8 times of gonocoxite length. Parameres fused forming V shaped structure. Aedeagus (Fig. 5) almost 1.5 times longer than its basal width, gradually narrowed apically.

Discussion. Indian specimens are larger in size than others of the world. Wings are wider, and both CR and AR are higher than those of others.

Distribution. China, Taiwan, New Guinea, Micronesia, Singapore, Australia and India (new record).

Bionomics and ecology. The above species are prevalent in different locations of West Bengal. The new species *Forcipomyia* (*Forcipomyia*) *hispida* sp. nov. got collected from the Dooars region (Jayanti), predominantly covered with long trees (*Shorea robusta*, *Michelia champaca*, *Schima wallichii*, *Lagerstroemia purviflora*, *Terminalia bellirica*, *Toona ciliata* etc.) at an altitude of 208 m. *Forcipomyia* (*Dycea*) *hamoni* was sampled from the Darjeeling Hills (Eastern Himalayas) at an elevation of 2078 m. Two other species, *Forcipomyia* (*Euprojoannisia*) *calamistrata* and *Forcipomyia* (*Euprojoannisia*) *fuscimana*, were collected from the Gangetic plains, at an altitude of 30–73 m.

Concluding remarks

In this article, one new member of the subgenus *Forcipomyia*, one new record of the subgenus *Dycea* Debenham and two new records of the subgenus *Euprojoannisia* Brèthes of the genus *Forcipomyia* Meigen are described from West Bengal, India. This study enriches the world catalogue of biting midges as well as it may help us to interpret new information on the biology of *Forcipomyia*.

DNA barcoding (Hebert et al. 2003) has been confirmed to be a valuable tool to delimit species of various taxa. Till date, the barcodes of very few Indian ceratopogonid species have been uploaded to databases. In this study, part of the mitochondrial gene COI (DNA barcode) of three male specimens of three different species (one newly described and two new records) have been sequenced, and the voucher specimens will be kept in the National Zoological Collection (ZSI) for future studies. These sequences may be helpful to resolve cryptic species complex within the group.

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